

Toxicity and Carcinogenicity Studies of Boric Acid in Male and Female B6C3F₁ Mice

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Toxicity and potential carcinogenicity studies of boric acid were investigated in mice to verify in a second rodent species that this was a noncarcinogenic chemical. Earlier chronic studies in rats indicated boric acid was not a carcinogen. The chemical is nominated for testing because over 200 tons are produced annually, there are multiple uses for the product, and there is potential for widespread human exposure, both orally and dermally. Both sexes of B6C3F₁ mice were offered diets mixed with boric acid for 14 days, 13 weeks, or 2 years. Dietary doses used in the acute, 14-day study were 0, 0.62, 1.25, 2.5, 5, and 10%; those in the subchronic, 13-week study were 0, 0.12, 0.25, 0.50, 1, and 2%; and doses in the 2-year, chronic study were 0, 0.25, and 0.50% in the diet. Mortality, clinical signs of toxicity, estimates of food consumption, body weight gain, and histopathologic examination of selected tissues constituted the variables measured. In the 14-day study mortality was proportional to dose and time of exposure in both sexes, occurring in dose groups as low as 2.5% and as early as 7 days of exposure. Body weights were depressed more than 10% below controls in the higher dose groups of both sexes. Mortality in the 13-week study was confined to the two highest dose groups in male mice and to the 2%-dose group in females. Body weight depression from 8 to 23% below those of controls occurred in the 0.50% and higher dose groups of both sexes. Minimal to mild extramedullary hematopoiesis in spleens of both sexes was a common occurrence in all dose groups, but the most severe lesion was testicular degeneration or atrophy of the seminiferous tubules in male mice fed 0.50 to 2.0% boric acid. Dietary doses of 0.25 and 0.50% were selected for both sexes of mice in 2-year studies based on body weights and mortality. Survival of male mice was reduced in the high-dose group after week 63, and after week 84 in the low-dose group; survival in female mice was not affected at either dose of boric acid. Reductions in body weight gain occurred in the high-dose group of both sexes. Based on estimates of food consumption, the average amount of boric acid ingested per mouse per day was calculated to be 400 to 500 mg/kg in the low-dose and 1100 to 1200 mg/kg in the high-dose groups of both sexes. Again the most prominent lesion was an increased incidence of testicular atrophy (3/49, 6/50, 27/47) and interstitial cell hyperplasia (0/49, 0/50, 7/47) in high-dose male mice. There was a slight increase in spleen lymphoid depletion in dosed male mice, which was considered secondary to stress and debilitation. Although there were marginal increases in subcutaneous tissue tumors and hepatic tumors in dosed male mice, these fell within the historical control range and were not believed to be related to chemical treatment. These particular tumors are highly variable in historical controls, only occurred in the low-dose group, and were not significant by an incidental tumor test, which is appropriate for tumors that are not a cause of death. There were also no indications that boric acid is genotoxic, since tests with prokaryotic and eukaryotic cells were uniformly negative, and there were no effects on sister-chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. — *Environ Health Perspect* 102(Suppl 7):93–97 (1994)

Key words: feeding study, 14-day repeated dose, 13-week subchronic, 2-year chronic, B6C3F₁ mice, testicular toxicity, carcinogen bioassay

Introduction

Boric acid occurs as the natural mineral sassolite in volcanic waters and hot springs, and has widespread commercial use in cosmetics, pharmaceuticals, and industrial processes (1). The major end uses for boric acid are in glass manufacturing and as a fire-retardant in cellulosic insulation (2).

A safe upper limit of 5% boric acid as an additive to cosmetics was recommended by the Cosmetic, Toiletry, and Fragrance Association (CTFA), 1983 (3). The U.S. Food and Drug Administration (FDA)

ruled that boric acid at $\leq 5\%$ was safe but ineffective as an ocular germicide, safe and effective as a buffer in ophthalmic preparations, but unsafe as a skin protectant, oral antimicrobial, and anorectal antiseptic, based on the absorption of boric acid by damaged skin and mucous membranes, its cumulative toxicity, and its slow elimination (4). The compound is registered as an indirect food additive for food packaging, and tolerances of 8 ppm total boron are permissible as citrus fruit residues (5). Boron is mildly irritating to the eyes and

mucous membranes, with a recommended threshold limit value (TLV) the same as that for other nuisance dusts of 10 mg/m³.

Although boric acid is classified as relatively nontoxic, with an acute oral dosage of LD₅₀ > 4 g/kg in rats (6), and subcutaneous dosage of > 1 g/kg in rats (7) and mice (8), mortality in humans has occurred after accidental ingestion, or application to abraded skin (9). These authors also cited clinical reports of central nervous system toxicity and toxicity to the genitourinary tract, liver, and skin in humans exposed to boric acid at pharmacologic concentrations.

The reproductive toxicity of boric acid was demonstrable in several rodent studies. Gavage doses of 1 g/kg per day in albino rats or dietary doses of 1.0% in Sprague-Dawley rats caused testicular atrophy after 14 days of gavage (10) or 90 days in the diet (6), with a no-effect level of about 100 mg/kg per day. Dietary doses of 700 mg/kg for 14 days resulted in mating

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These studies were performed as part of the National Toxicology Program (NTP) tests of chemicals to evaluate toxicity and carcinogenicity. Contributors included staff from the NTP who designed and monitored the studies, evaluated the experiments, interpreted the results, and reported the findings as NTP Technical Report No. 324; staff from EG&G Mason Research Institute who conducted the studies and evaluated tissues; an NTP Pathology Working Group that evaluated the slides and prepared a pathology report; staff from the Experimental Pathology Laboratories, Inc. who provided pathology quality assurance; and staff from Caritech Associates, Inc., who provided support for the preparation of the technical report.

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sterility in Sprague-Dawley rats, causing spermatotoxicity in males and decreased ovulation in females, but lower doses of 67 and 200 mg/kg per day did not affect reproduction through three generations (6). Ingestion of 6 mg/l of boron equivalents per day for 6 months also caused testicular atrophy in rats (11).

Ingestion of boric acid resulted in its accumulation in brain, liver, and fat, and its excretion into the urine, feces, saliva, milk, and perspiration (12). Apparently this compound is poorly absorbed through intact skin, since there was no chemical residue in sera of females treated twice daily for 14 days with vaginal inserts of 600 mg boric acid. However, in infants with diaper rash or burns (7,13,14) or adults with burns (15,16) or vaginitis (17), boric acid absorption occurred through the compromised dermal layer, and appeared in the urine.

The Consumer Product Safety Commission and the U.S. Environmental Protection Agency nominated the chemical for testing by the National Toxicology Program (NTP) because over 200 tons are produced annually (2); there are multiple uses for the product (3), and there is potential for widespread human exposure orally and dermally (1,4,18). Earlier chronic studies in Sprague-Dawley rats indicated boric acid is not a carcinogen (6). Additional prechronic and chronic studies of boric acid were conducted in mice to investigate the toxicity of this widely used product, and to verify in a second rodent species that this is a noncarcinogenic chemical.

Materials and Methods

Technical-grade boric acid was obtained in one batch from Thompson Haywood Chemicals (Kansas City, MO). The substance was confirmed to be boric acid by elemental analysis and infrared and ultraviolet/visible spectroscopy by Midwest Research Institute (Kansas City, MO). Cumulative data indicated the boric acid was approximately 99.7% pure, and was stable for 2 weeks at 60°C. Periodic characterization of the boric acid study material and a reference standard indicated no degradation over the course of the studies. Dose mixes were prepared with National Institute of Health (NIH) 07 rat and mouse ration, using a Patterson-Kelly V-blender with intensifier bar, and were stored at 0° ± 5°C for no longer than 2 weeks. Periodic analyses indicated the dose mixtures were within 10% of target concentrations.

Male and female B6C3F₁ mice were obtained from Charles River Breeding Laboratories for the 14-day and 13-week studies, and from Frederick Cancer Research Center (Frederick, MD) for the 2-year study. Animals were checked for health status and quarantined for 2 to 3 weeks before studies. They were randomized according to body weight, identified, and placed on studies at 7 to 9 weeks of age. Animals were maintained five to a cage in polycarbonate containers with nonwoven filters on Aspen Bed bedding, and were given feed *ad libitum*, and automatic watering. The animal room environment was maintained at 20.0° to 28.9°C, 11 to 79% humidity, with fluorescent lighting 12 hr/day, and with 10 to 12 room air changes per hour.

Both sexes of B6C3F₁ mice were offered untreated food or diets mixed with analytical grade boric acid for 14 days, 13 weeks, or 2 years. Doses used ranged from 0.62 to 10.0% of the diet in 14-day studies, from 0.12 to 2.0% in 13-week studies; doses were 0.25 and 0.50% in the 2-year chronic studies. Mortality, clinical signs of toxicity, estimates of food consumption, body weight gain, selected organ weights, and histopathologic examination of selected tissues constituted the variables examined.

Statistical treatment of tumor incidence data included life table analyses (19,20) and incidental tumor analyses (21). A more complete discussion of these methods can be found in NTP Technical Report on boric acid (22).

Results

In the 14-day studies 5/5, 3/5, and 1/5 male mice ingesting 10, 5, and 2.5% boric acid in the diet, respectively, died as early as day 7 of exposure; 4/5 female mice died between days 9 and 11 (Table 1). Hyperplasia of the forestomach was the most prominent lesion observed. There was no apparent effect on food consumption, but body weights were depressed more than 10% below controls in males fed 5 or 2.5%, and in females fed 10% boric acid in the diets.

Mortality in the 13-week studies was confined to the 1 and 2%-dose groups, where 8/10 males and 6/10 females fed 2% boric acid died between weeks 1 and 8; one male mouse in the 1%-dose group died at week 2 of exposure (Table 2). There was no apparent effect on food consumption, although body weight depression of 10 to 23% below controls occurred in males fed 0.50, 1.0, and 2.0% boric acid, and of 8 to 18% in female mice from the same dose groups. Minimal to mild extramedullary hematopoiesis in the spleens of both sexes was a common occurrence in all dose groups, and hyperkeratosis and/or hyperplasia of the forestomach also occurred in the high-dose group of both sexes; but the most severe lesion was testicular degeneration or atrophy of the seminiferous tubules in male mice fed 0.50 to 2.0% boric acid (Table 3).

Dietary doses of 0.25 and 0.50% were selected for both sexes of mice in 2-year studies based on body weight depression and

Table 1. Mortality and body weights in male and female B6CF₁ mice fed boric acid for 2 weeks.

Dose %	Mortality		Body weight, g	
	Males	Females	Males	Females
0	0/5	0/5	29.9 ± 0.9	22.3 ± 0.8
0.62	0/5	0/5	28.4 ± 1.1	22.0 ± 0.9
1.25	0/5	0/5	28.8 ± 0.9	21.3 ± 0.3
2.5	1/5 ^a	0/5	26.3 ± 1.4	20.7 ± 0.9
5.0	3/5 ^b	0/5	24.6 ± 0.2	21.3 ± 0.8
10.0	5/5 ^c	4/5 ^d	—	18.4

Day at death: ^a 13; ^b 8, 14, 14; ^c 7, 7, 8, 13, 14; ^d 9, 11, 11, 11.

Table 2. Mortality and body weights in male and female B6C3F₁ mice fed boric acid for 13 weeks.

Dose, %	Mortality		Body weight, g	
	Males	Females	Males	Females
0	0/10	0/10	38.0	29.8
0.12	0/10	0/10	36.8	28.5
0.25	0/10	0/10	37.2	28.5
0.50	0/10	0/10	34.0	27.5
1.0	1/10 ^a	0/10	31.5	26.7
2.0	8/10 ^b	6/10 ^c	29.1	24.3

Week at death: ^a 2; ^b 1, 2, 2, 2, 3, 3, 6, 8; ^c 1, 2, 3, 3, 5, 6

Table 3. Incidence of nonneoplastic lesions in male and female B6C3F₁ mice fed boric acid for 13 weeks.

Site/lesion	Dose, percent in feed					
	0	0.12	0.25	0.50	1.0	2.0
Male						
Spleen, extramedullary hematopoiesis	1/10	3/10	5/10	5/10	10/10	1/10
Forestomach, hyperkeratosis and/or hyperplasia	0/10	0/10	0/10	0/10	0/10	8/10
Testis, degeneration/atrophy of seminiferous tubules	0/10	0/10	0/10	2/10	8/10	8/10
Female						
Spleen, extramedullary hematopoiesis	0/10	2/10	4/10	6/10	10/10	2/10
Forestomach, hyperkeratosis and/or hyperplasia	0/10	0/10	0/10	0/10	0/10	3/10

mortality in mice fed 1.0 and 2.0% boric acid for 13 weeks. Survival rates of male mice in the 2-year study fell in the high-dose group after week 63, and after week 84 in the low-dose group. Survival in female mice was not affected at either dose of boric acid (Figure 1). There were no apparent effects on food consumption, but reductions in body weight gain occurred in the high-dose group of both sexes (Figure 2).

Again the most prominent nonneoplastic lesion was an increased incidence of testicular atrophy (3/49, 6/50, and 27/47) and interstitial cell hyperplasia (0/49, 0/50, and 7/47) in control-, low-, and high-dose male mice (Table 4). The lesion was characterized by a variable loss of all stages of spermatocytes, with the seminiferous tubules containing mainly Sertoli cells, and in

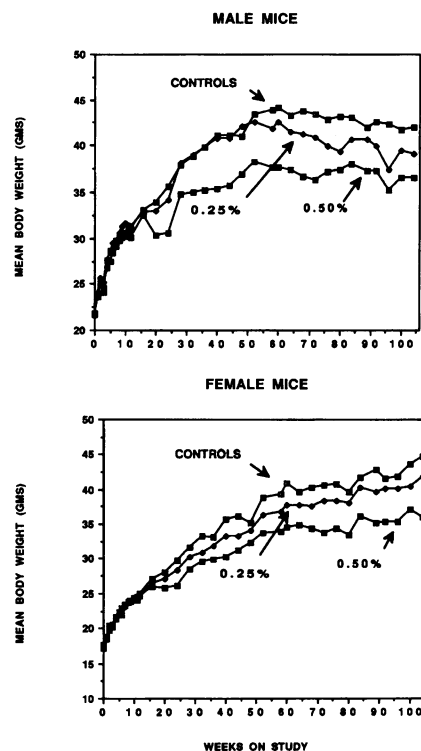
some mice, an increased number of interstitial cells.

There were marginal increases in hepatic tumors and subcutaneous tissue tumors in dosed male mice, but these were not believed to be related to chemical treatment (Table 5). The combined incidence of hepatocellular adenomas or carcinomas were 14/50, 19/50, and 15/49 in the male mice fed 0, 0.25, or 0.50% boric acid, respectively, for 2 years. The combined incidences of sarcomas, fibrosarcomas, or neurofibrosarcomas in the same-dose groups of male mice were 2/50, 10/50, and 2/50. None of these tumor incidences, nor any other kinds of tumors, were increased in female mice.

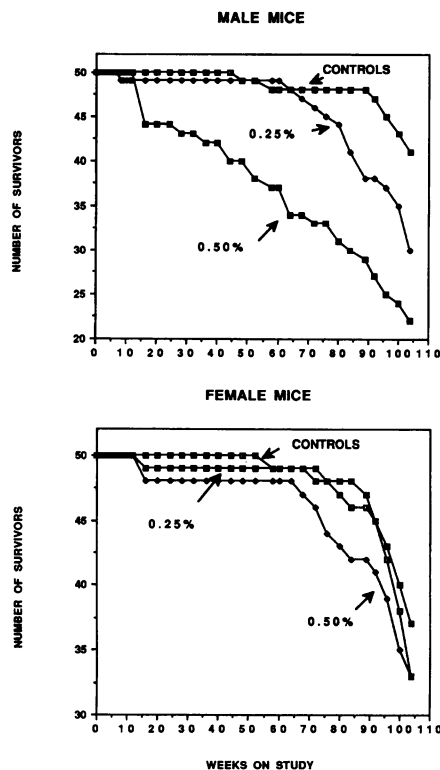
Mutagenicity assays with *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were negative in the presence or absence of S9 fractions prepared from livers of Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters. Additional tests in the mouse lymphoma L5178Y/TK +/– assay with or without activation by S9 from Aroclor 1254-induced male F344 rat liver were also negative. There were no effects of boric acid on sister-chromatid exchanges or on chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9 fractions from Aroclor 1254-induced male Sprague-Dawley rat liver (22).

Discussion

The 0.25 and 0.50% doses selected for the 2-year study in mice were good approximations for a maximum-tolerated dose and one-half that dose. After about week 30 a

**Figure 2.** Growth curves for male and female B6C3F₁ mice fed diets containing boric acid for 2 years.

dose-related reduction in body weight gain occurred in both mice sexes. At study termination average body weights of survivors were 42.0, 39.1, and 36.5 g in control-, low-, and high-dose males, and 44.8, 41.8, and 35.9 g in the same female dose groups. Unpalatability of the boric acid diets could account for some of the decrease in body weight gain, but spillage was also a factor. Although food consumption appeared to decrease in chemically treated groups, inanition was probably not a cause of death, since excess mortality occurred only in male mice from the high-dose group. Survival in male mice was reduced after week 63 in the high-dose, and after week 84 in the low-dose groups, but since five accidental deaths occurred in male mice from the high-dose group, these differences

**Figure 1.** Kaplan-Meier survival curves for male and female B6C3F₁ mice fed diets containing boric acid for 2 years.**Table 4.** Incidence of nonneoplastic lesions in male B6C3F₁ mice fed boric acid for 2 years.

Site/lesion	Dose, percent in feed		
	0	0.25	0.50
Spleen			
Lymphoid depletion	5/48	11/49	25/48
Testis			
Degeneration/atrophy of seminiferous tubules	3/49	6/50	27/47
Interstitial cell hyperplasia	0/49	0/50	7/47

Table 5. Analysis of tumors in male B6C3F₁ mice fed boric acid for 2 years.

Site/lesion	Dose, percent in feed		
	0	0.25	0.50
Hepatocellular adenoma or carcinoma ^a			
Overall rates	14/50	19/50	15/49
Adjusted rates	31.4%	51.4%	50.4%
Terminal rates	11/41 (21%)	13/30 (43%)	8/22 (36%)
Week of first observation	92	65	43
Life table tests	$p = 0.023$	$p = 0.043$	$p = 0.038$
Incidental tumor tests	$p = 0.183$	$p = 0.211$	$p = 0.259$
Fibroma, sarcoma, fibrosarcoma, or neurofibrosarcoma ^b			
Overall rates	2/50	10/50	2/50
Adjusted rates	4.9%	26.6%	7.1%
Terminal rates	2/41 (5%)	4/30 (13%)	1/22 (5%)
Week of first observation	104	82	54
Life table tests	$p = 0.239$	$p = 0.005$	$p = 0.493$
Incidental tumor tests	$p = 0.491$	$p = 0.026$	$p = 0.676$

^a Historical incidence at contract laboratory (mean \pm SD): 216/697 (31% \pm 9%). Historical incidence in National Toxicology Program studies: 62/2084 (30% \pm 8%). ^b Historical incidence at contract laboratory (mean \pm SD): 39/697 (6% \pm 4%). Historical incidence in National Toxicology Program studies: 156/2091 (7% \pm 8%).

were probably not attributable to chemical treatment. At study termination there were 41, 30, and 22 male mice; and 33, 33, and 37 female mice in control-, low-, and high-dose groups available for histologic examination. The numbers of females were sufficient to detect the potential toxicologic effects of boric acid, but the lower numbers of males may have reduced the sensitivity of the study.

Just as in the prechronic studies, in the 2-year study, testicular atrophy and interstitial cell hyperplasia in male mice were the predominant lesions related to chemical treatment. Atrophy of the seminiferous tubules was observed in more than half of the male mice dosed with 0.50% boric acid, but mice in the 0.25%-dose groups were unaffected. Similarly, interstitial cell hyperplasia occurred in 7/47 male mice in the high-dose group, but not at all in mice from the lower

dose group, indicating a narrow threshold of male reproductive toxicity for boric acid. The toxic effects of boric acid on the testis have been well documented in other rodent studies (6,10,11). Although testicular toxicity represents the major hazard from this chemical, these studies used either very high doses (1000 mg/kg for 14 or 90 days), or very long durations of exposure (6 mg/l in drinking water for 6 months). It is unlikely that cosmetics that contain 0.10 to 5.0% boric acid (3), or that nuisance dust levels from occupational exposure (10 mg/m³ \sim 1 mg/kg/day in humans) would constitute a risk of reproductive toxicity in humans. For example, since mice normally eat about 4 g of food per day, best estimates of chemical consumption for those given 0.25 and 0.50% boric acid for 2 years in the present study were about 275 and 550 mg/kg/day, with a no-effect level for testicular toxicity

between these values. Comparable reproductive toxicity in male rats required about the same concentrations of boric acid in the diet, and a no-effect level of 100 mg/kg/day was noted (6).

Slight increases in the incidences of subcutaneous tissue tumors and hepatocellular tumors occurred in the low-dose group of male mice, but not in the high-dose group, nor in either dose group of female mice. Several factors indicated the occurrence of these tumors was not related to the administration of boric acid. There was a lack of a relationship between tumor incidence and dose. The tumors occurred in tissues that were not target organs, and not in the testes, the most sensitive site identified for target organ toxicity, nor in other parts of the reproductive tracts of either sex. The increased tumor incidences were significantly different from concurrent controls only when calculated by a life table test, which is inappropriate for tumors that are not a cause of death. In addition, the incidences of both of these neoplasms are highly variable, and each fell within the range of reported historical control values. There were also no indications that boric acid acted as a genotoxic agent to induce cancer, since tests with prokaryotic and eukaryotic cells were uniformly negative, and there were no effects on sister chromatid exchanges or on chromosomal aberrations in Chinese hamster ovary cells (22,23).

An earlier 2-year study in Sprague-Dawley rats showed that boric acid was not carcinogenic in either sex (6). The present study confirmed this result in a second species, showing that boric acid was also not a carcinogen in either sex of B6C3F₁ mice.

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